U.S. National Stage Application of PCT/EP03/04297

Attorney Docket No.: 53262-20092.00

b.) Amendments to the Specification

Please amend the specification as follows (page and line numbers refer to the enclosed English language version of the application):

- At page 1, line 5, please delete:
- "Description" and insert therefore -- Field of the Invention --
- At page 1, line 25, please insert:
 - -- Description of the Background --
- At page 5, line 2, please insert:
 - -- Summary of the Invention

The present invention provides, generally, methods for production of polyunsaturated acids in plants.

One embodiment of the invention is directed to processes for the production of compounds in transgenic plants wherein the compounds comprise the formula of Formula I:

wherein: R¹ = -OH, coenzyme A (thioester), phosphatidylcholine, phosphatidylethanolamine, phoshatidylglycerol, diphosphatidylglycerol, phosphatidylserine, phosphatidylinositol, sphingolipid, glycoshingolipid or a radical of Formula II;

$$\begin{array}{c|c}
H_2C-O-R^2 \\
HC-O-R^3 \\
H_2C-O
\end{array}$$
(II)

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wherein R^2 = H, phosphatidylcholine-, phosphatidylethanolamine-, phosphatidylglycerol-, diphosphatidylglycerol-, phosphatidylserine-, phosphatidylinositol-, shingolipid-, glycoshingolipid- or saturated or unsaturated C_2 – C_{24} –alkylcarbonyl-; and R^3 = H, saturated or unsaturated C_2 – C_{24} –alkylcarbonyl-. Preferably, R^2 and R^3 are independent of one another and represent a radical of Formula Ia:

$$\begin{array}{c|c}
CH_{2} & CH_{2} \\
\hline
CH_{2} & CH_{2}
\end{array}$$

$$\begin{array}{c|c}
CH_{2} & CH_{2} \\
\hline
CH_{2} & CH_{3}
\end{array}$$
(Ia),

wherein n = 3, 4 or 6; m = 3, 4 or 5; and p = 0 or 3.

The compounds preferably have a content in the transgenic plant of at least 1% by weight based on the total fatty acid content of the plant or, more preferably, 5% by weight. The process generally comprises introducing into a plant: at least one first nucleic acid sequence which encodes a polypeptide with an $\Delta 6$ -desaturase activity; at least one second nucleic acid sequence which encodes a polypeptide with a $\Delta 6$ -elongase activity; and, optionally, at least one third nucleic acid sequence which encodes a polypeptide with a $\Delta 5$ -desaturase activity. The process further comprises growing and harvesting the transgenic plants.

Preferably, variables R^2 and R^3 are independent of one another and are C_{10} - C_{22} -alkylcarbonyl-, or are C_{16} -, C_{18} -, C_{20} - or C_{22} -alkylcarbonyl-, wherein any of the preceding may have one, two, three, four or five double bonds. Preferably, the plant is a plant of an oil crop, or a plant selected from the group soya, peanut, oilseed rape, canola, linseed, evening primrose, verbascum, thistle, hazelnut, almond, macadamia, avocado, bay, wild roses, pumpkin/squash, pistachios, sesame, sunflower, safflower, borage, maize, poppy, mustard, hemp, castor-oil plant, olive, Calendula, Punica, oil palm, walnut and coconut.

Preferably, the compounds are obtained from the oils, fats, lipids or free fatty acids of the transgenic plants by pressing or extraction, and once obtained may be further

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refined. Also preferably, the saturated or unsaturated fatty acids present in the compounds may be liberated such as, for example, by alkaline hydrolysis or enzymatic cleavage.

Preferably, one or more of the nucleic acid sequences which encode the polypeptides with $\Delta 6$ -desaturase activity, $\Delta 6$ -elongase activity or $\Delta 5$ -desaturase activity, may be selected from the group consisting of: a) a nucleic acid sequence with the sequence shown in SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 23, SEQ ID NO: 25, SEQ ID NO: 27, SEQ ID NO: 29 or SEQ ID NO: 31, b) nucleic acid sequences which, owing to the degeneracy of the genetic code, are obtained by back translation of the amino acid sequences shown in SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14, SEQ ID NO: 16, SEQ ID NO: 18, SEQ ID NO: 20, SEQ ID NO: 22, SEQ ID NO: 24, SEQ ID NO: 26, SEQ ID NO: 28, SEQ ID NO: 30 or SEQ ID NO: 32, or c) derivatives of the nucleic acid sequences shown in SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 23, SEQ ID NO: 25, SEQ ID NO: 27, SEQ ID NO: 29 or SEQ ID NO: 31 which encode polypeptides with the amino acid sequences shown in SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14, SEQ ID NO: 16, SEQ ID NO: 18, SEQ ID NO: 20, SEQ ID NO: 22, SEQ ID NO: 24, SEQ ID NO: 26, SEQ ID NO: 28, SEQ ID NO: 30 or SEQ ID NO: 32 and which have at least 50% homology at the amino acid level, without the enzymatic activity of the polypeptide being substantially reduced. Nucleic acid sequences may be linked with one or more regulatory signals in a nucleic acid construct and, nucleic acid constructs may additional biosynthetic genes of the fatty acid or lipid metabolism such as, preferably, acyl-CoA dehydrogenase(s), acyl-ACP [= acyl carrier protein] desaturase(s), acyl-ACP thioesterase(s), fatty acid acyl transferase(s), fatty acid synthase(s), fatty acid

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hydroxylase(s), acetyl-coenzyme A carboxylase(s), acyl-coenzyme A oxidase(s), fatty acid desaturase(s), fatty acid acetylenases, lipoxygenases, triacylglycerol lipases, allene oxide synthases, hydroperoxide lyases or fatty acid elongase(s).

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Other embodiments and advantages of the invention are set forth in part in the description, which follows, and in part, may be obvious from this description, or may be learned from the practice of the invention.

Description of the Drawings

- Figure 1. Biosynthesis chain.
- Figure 2. Fatty acid profile of transgenic tobacco seeds.
- Figure 3. Fatty acid profile of wild tobacco seeds as control.

Description of the Invention --

- At page 54, line 35, please insert:
- -- Other embodiments and advantages of the invention are set forth in part in the description, which follows, and in part, may be obvious from this description, or may be learned from the practice of the invention. All references cited herein, including all U.S. and foreign patents and patent applications, and all publications or other documentary materials, are specifically and entirely hereby incorporated herein by reference. It is intended that the specification and examples be considered exemplary only. --